BIOLOGICAL FEATURES OF HUMAN T-ACTIVATED KILLER CELLS

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ABSTRACT

Objective: To investigate the immunobiological essence of T-activated killer (T-AK) cells induced by anti-CD_3 monoclonal antibody (CD_3McAb) and recombinant interleukin-2 (rIL-2) co-stimulation. Methods: The cytomorphology, phenotype and cytotoxicity of T-AK cells generated from human peripheral blood mononuclear cells (PBMC) were determined. Results: T-AK cells were similar to activated lymphoblasts in morphology, more than 90% of T-AK cells expressed the phenotypes of T-lymphocytes (CD_3^+, CD_8^+), and 20%~50% of the cells were NK-like phenotype (CD_{16}^+, CD_{56}^+), some of them expressed IL-2 receptor (CD_{25}^+), CD_{38} antigen (CD_{38}^+) and MHC-II antigen (HLA-DR^+) characteristic marks for the activated T lymphocytes. T-AK cells attacking targets were morphologically large volumes with granules and mainly contained CD_8^+ and CD_{56}^+ cells. T-AK cells possessed high tumoricidal activities against NK-sensitive K_{562} cells and NK-resistant Raji cells, the cytotoxicity was composed of mainly CD_3McAb-activated CD_3AK activity (~50%), IL-2 induced LAK activity (~30%), NK activity (~10%) and the activities of inhibitory factors in T-AK supernatant (~10%). Conclusion: T-AK cells are a heterogeneous cell population consisting of mainly activated T lymphocytes and NK-like cells, the main part of T-AK cytotoxicity is the common activities of CD_3AK cells and LAK cells.

Key words: T-activated killer (T-AK) cell, Cytomorphology, Phenotype, Cytotoxicity, Heterogeneity

Cancer biotherapy needs to generate tumor-killing effectors *in vitro*, but different tumoricidal activities are induced from peripheral subpopulation of lymphocytes depending on the different stimuli used to activate the cells. Previous reports[1-4] have found that NK cells are primarily responsible for the lytic function in interleukin-2 (IL-2)-induced LAK (lymphokine-activated killer) cells, while T-cells, if present, exhibit little if any cytotoxicity. The addition of anti-CD_3 monoclonal antibody (CD_3McAb) or/and low concentration of IL-2 (<100U/ml) generates cells with lytic activity which are predominantly CD_3^+lyt-2^+ T-cells ($CD_3^+CD_8^+$ T-cells in human). CD_3McAb in combination with a high concentration of IL-2 co-stimulated T-AK (T-activated killer) cells containing T-lymphocytes as well as NK cells, and their *in vitro* and *in vivo* antitumor effects are much higher than those of tumor-killing effectors induced by IL-2 or CD_3McAb alone.[1,4,8] The present work describes systemically the cytomorphology, phenotypes, cytotoxic components of T-AK cells induced with CD_3McAb and rIL-2 as a stimulus from human peripheral blood monuclear cells (PBMC).

MATERIALS AND METHODS

Reagents

CD_3McAb and rIL-2 were purchased from the Academy of Military Medical Sciences, PLA; MTT was from Sigma Corporation, and sodium dodecyl sulfate (SDS) from Serva Corporation; Serial CD monoclonal antibodies, anti-HLA-DR antibody and APAAP-kit were purchased from Institute of Hematology, Chinese Academy of Medical Sciences and Beijing BioTinge Biomedicine Co., respectively.

Target Cells

K_{562} cells (human leukemia cell line) and Raji cells (burkitt's lymphoma cells) were preserved in tumor cell bank of our institute.

Generation and Cytotoxicity Analyses of T-AK

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Cells

As previously described.[1,6,8] Briefly, PBMC was separated from human peripheral blood by Ficoll-Hypaque density gradient centrifugation (d=1.077), and incubated at 2×10^6 cells/ml in complete RPMI 1640 culture media (TCM) in the presence of 1.0 μg/ml CD_3McAb plus 200 units/ml rIL-2 for generating T-AK cells. To evaluate the lytic activity of the different cell subpopulations within T-AK cell population, CD_3McAb (1.0 μg/ml) or rIL-2 (200 units/ml) was added only in TCM for inducting CD_3AK activity and LAK activity separately; PBMC was cultured in TCM without rIL-2 or CD_3McAb as effectors in determining NK activity in T-AK cells. All the cells were induced and expanded for 72 hours to 120 hours, and served as effectors to examine the cytolytic activities against K_{562} and Raji target cells by an improved MTT colorimetric assay.[6]

Preparation and Tumor-Inhibitory Activities of T-AK Supernatant

T-AK cells and K_{562} cells were mixed at a ratio of 20:1 and incubated for 24 hours, then the supernatant was separated by centrifugation, its inhibitory activity on K_{562} cells and Raji cells was determined.[7] Cell sediment (T-AK cells and K_{562} cells) was smeared to observe the morphology and phenotypes of T-AK cells attacking K_{562} cells.

Cytomorphological Examination of T-AK Cells

A cell smear centrifuge (TP-2 type, Zhenzhou Medical Appliance Factory, China) made T-AK cell smears, and they were stained with Wright-Giemsa stain. The cellular and nuclear diameters of 20 cells were measured randomly with a micrometer (Jiangsu Xinming Optical Instrument Factory, China. Eyepiece 1/10 mm and object 1/100mm), and the ratios of cellular and nuclear diameters were calculated. The morphological characteristics of active and stained cells were described.

Phenotypic Analysis of T-AK Cells

T-AK cells were collected, washed and smeared by centrifugation, and stained with an APAAP immunohistochemical procedure.[5] The data were expressed as the percentages of positive cells (%).

RESULTS

Morphological Characteristics of T-AK Cells

In the presence of CD_3McAb and rIL-2 the volumes of the stimulated cells enlarged gradually, and a ball-like "colony" mainly consisting of large-volumed cells was formed under an inverted microscope. Activated T-AK cells, if stained, were moderately different in shapes and sizes, but most of them were large-volume cells (14~28 μm in diameter). T-AK cells owned round or elliptical shapes with granules in their rich cytoplasms, the nuclear chromatin was loosed like network and the nucleoli were large and clear. The nucleus/cytoplasm ratio was markedly decreased (Table 1). The phenomena of cell division were easy to find in the smear. T-AK cells gathering around and attaching K_{562} cells were morphologically large volume with granules and surrounded targets closely, some of them inlaid into the cytoplasm of K_{562} targets. The chromatin of K_{562} cell attached was concentrated and the cytoplasm was vacuolated totally.

Table 1. Morphology of T-AK cells (induced for 120 hours and 360 hours, n=20 $\bar{x}\pm s$)

Cells	Cell diameter (μm)	Nucleus/cytoplasm
PBMC	9.20 ± 1.73	0.88 ± 0.07
T-AK (120)	19.18± 3.46*	0.73 ± 0.06*
T-AK(360)	20.71 ± 2.54*	0.71 ± 0.05*

Note: * $P<0.01$ compared with PBMC

Phenotype of T-AK Cells

After induced and expanded for 240 hours, about 90% of T-AK cell were phenotypes of T lymphocytes (CD_3^+ CD_8^+), 20% to 50% of the cells were NK-like phenotypes (CD_{16}^+ CD_{56}^+), and some of T-AK cells expressed CD_{38} antigen (CD_{38}^+), IL-2R (CD_{25}^+) and MHC II antigens ($HLA\text{-}DR^+$) characteristic for the activated T lymphocytes (Table 2). In the process of killing target cells, the T-AK cells attaching K_{562} cells mainly contained CD_8^+ and CD_{56}^+ cells.

Table 2. Phenotypes of T-AK cells (n=3, $\bar{x} \pm s$)

Phenotypes	Positive cells (%)
CD2	83.88 ± 3.17
CD3	92.67 ± 2.62
CD4	49.34 ± 3.40
CD8	90.89 ± 2.49
CD16	22.17 ± 3.27
CD25	40.67 ± 1.89
CD38	96.18 ± 1.43
CD56	46.83 ± 12.49
HLA-DR	63.77 ± 3.54

Cytotoxicity of T-AK Cells

After activation with CD_3McAb plus rIL-2, the cytolytic activity of T-AK cells against NK-sensitive K_{562} and NK-resistant Raji targets was tested, and each activity induced by different stimulating substances in T-AK culture system was determined

separately. Analysis of the lytic function of T-AK culture demonstrated that T-AK cytotoxicity was composed of several cytolytic components: CD_3McAb-activated CD_3AK activity was response for approximately 50% of the cytolytic activity of T-AK cells; IL-2-induced LAK activity, about 30%; NK activity roughly 10%; the inhibitory activity of the inhibitory factors released in the process of T-AK cells killing targets, around 10% (Table 3, Table 4). As shown in Table 3 and Table 4, the proportion of each activity in that of T-AK cells was slightly different along with prolonging the stimulating culture period and changing the target cells. The total percentage of separately assessed activities of the four effectors was higher than that of T-AK cytotoxicity; this was probably related to the partly overlapping of some activities (such as LAK and NK) and the measuring errors of the separate determination.

Table 3. The components of the cytotoxicity of T-AK cells induced for different times (K_{562} cells as target cells, effector: target=20:1. n=4, $\bar{x} \pm s$)

Effectors	Cytotoxicity(%)	
	72 h	120 h
T-AK	96.43±3.04	97.12±2.44
CD_3AK	61.67±10.12(0.46)	84.00±5.87(0.56)
LAK	34.14±5.04(0.25)	41.18±8.85(0.27)
NK	22.26±4.66(0.17)	9.24±3.06(0.06)
Supernatant	16.28±6. 21(0.12)	16.28±6.21(0.11)
Sum of 4activities	134.35(1.00)	150.70 (1.00)

Note: The numbers in the parentheses show the activity proportion of each effector in the sum of the activities of the four effectors in T-AK cytotoxicity

Table 4. The components of the cytotoxicity of T-AK cells against different target cells (induced for 120 hours, effector: target=20:1. N=4, $\bar{x} \pm s$)

Effectors	Cytotoxicity(%)	
	K_{562}	Raji
T-AK	89.86±10.04	78.13±6.67
CD_3AK	48.67±2.18(0.50)	40.00±7.89(0.49)
LAK	28.18±4.87(0.29)	32.00±2.24(0.39)
NK	9.24±1.04(0.10)	1.87±0.97(0.02)
Supernatant	10.47±3.34(0.11)	8.48±2.19(0.10)
Sum of 4 activities	96.56 (1.00)	82.35 (1.00)

Note: The numbers in the parentheses show the activity proportion of each effector in the sum of the activities of the four effectors in T-AK cytotoxicity.

DISCUSSION

T-AK cells are tumoricidal cells with high cytolytic activity generated by CD_3McAb and rIL-2 co-activation.[1-4,6-8] Our results confirmed that T-AK cells were morphologically similar to those of lymphoblasts, their main phenotypes were those of T lymphocytes (CD_3^+ CD_8^+), the next was CD_{16}^+ CD_{56}^+ NK-like phenotypes, and some of them expressed characteristic marks (IL-2R, CD_{38} and MHC II antigens, etc.) for the activated T lymphocytes. Previous studies showed that the utilization of CD_3McAb or rIL-2 as a stimulus had different actions on different cell populations. CD_3McAb mainly activated T lymphocytes, while rIL-2 started the activation of NK cells as well as T-cells. Therefore T-AK cells are provided with some common biological features of CD_3McAb-activated CD_3AK cells and rIL-2-induced LAK cells.[1,3,7]

The heterogeneity is present in the cytotoxicity of T-AK cells. This paper utilizing a method of separate generation and determination confirmed that cytolytic activity of T-AK cells against targets consisted of at least four parts: NK-sensitive K_{562} cells served as targets, the component of T-AK cytotoxicity was CD_3McAb-activated CD_3AK activity (~50%), rIL-2-induced LAK activity (~30%), NK activity (~10%) and activities of inhibitory factors in the T-AK supernatant (~10%). NK-resistant Raji cells as targets CD_3AK activity, LAK activity and inhibitory factor were responsible for approximately 50%, 40% and 10% of the lytic function of T-AK cells, respectively, and NK activity was consistently less than 2%. The heterogeneity of T-AK cell populations was confirmed in the functions combined with the morphology and the phenotypes.

With the heterogeneity expressed in the cytomorphology, phenotypes and cytotoxicity of T-AK cells, we have clearly demonstrated that T-AK cells are not an independent cell population or subset. These cells are a heterogeneous cell population with high cytolytic activity, the main part of T-AK cells is activated T lymphocytes and NK cells, and they possess the co-activities of CD_3AK cells and LAK cells.

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